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IS*Ecp1B*-Mediated Transposition of bla_{CTX-M} in *Escherichia coli*

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Several expanded-spectrum β-lactamase bla_{CTX-M} genes are associated with IS*Ecp1*-like elements in *Enterobacteriaceae*. We found that IS*Ecp1B* was able to mobilize the adjacent $bla_{CTX-M-19}$ gene by a transpositional mechanism in *Escherichia coli* by recognizing a variety of DNA sequences as right inverted repeats.

An increasing number of reports of expanded-spectrum β-lactamases of the CTX-M type and plasmid-mediated cephalosporinases of the AmpC type in *Enterobacteriaceae* raise the question of their acquisition (5, 24, 26). The bla_{CTX-M} genes belonging to three out of the five known clusters (the CTX-M-1, -M-2, and -M-9 clusters) are associated with IS*Ecp1*-like insertion sequences (IS) (2, 5–7, 11, 12, 18, 21, 23). Nevertheless, the distance that separates the β-lactamase gene from IS*Ecp1* varies within a given cluster of CTX-M-type genes, indicating that different insertion events have occurred (13, 19). Analysis of GenBank databases reveals that some plasmid-encoded cephalosporinase genes, such as the bla_{CMY}-type genes, may also be associated with IS*Ecp1* (3, 10, 17).

IS*Ecp1* is an IS weakly related to other IS elements and belonging to the IS*1380* family (IS Database HomePage [http://www-is.biotoul.fr/page-is.html]) (8). Nothing is known about the mobilization properties of IS*Ecp1*. Recently, we characterized IS*Ecp1B* associated with a $bla_{CTX-M-19}$ gene in a *Klebsiella pneumoniae* ILT-3 clinical isolate from Vietnam (21). IS*Ecp1B* differs from IS*Ecp1* (GenBank accession no. AJ242809) by three nucleotide substitutions. Their inverted repeat (IR) sequences are identical, and their transposases differ by a single amino acid change (18). Since IS*Ecp1*-like elements are located upstream of several β-lactamase genes, analysis of the variable sequences separating these IS elements from the initiation codons of these genes allowed us to determine its boundaries.

Recently, it has been shown that IS*Ecp1B* brought promoter sequences for high-level expression of the $bla_{CTX-M-14/-18}$, $bla_{CTX-M-17}$, and $bla_{CTX-M-19}$ β-lactamase genes that belong to the same $bla_{CTX-M-9}$ subgroup (5, 6, 18) and might be also responsible for their mobilization. The aim of this study was to analyze the mobilization process mediated by IS*Ecp1B*.

IS*Ecp1B* possesses two imperfect IRs likely made of 14 bp, previously estimated to be 18 bp (18), with 12 of these 14 bp being complementary (Table 1). A detailed analysis of a ca. 9.5-kb DNA fragment from natural plasmid pILT-3 from *K. pneumoniae* ILT-3 revealed that the gene encoding ORF1 in transposon Tn*1721* was interrupted by a 4.8-kb potential transposon bracketed on the left side by the left IR (IRL) of IS*Ecp1B* and on the right side by an imperfect right IR (IRR) named *IRR1* made of a 14-bp sequence belonging to a gene encoding a putative iron transport protein (Fig. 1) (18). This potential transposon comprises IS*Ecp1B*, $bla_{CTX-M-19}$, IS*903D*, and part of the gene encoding an iron transport protein. We have hypothesized that IS*Ecp1B* could mobilize adjacent sequences by transposition.

Several transposition experiments were performed to determine (i) the mobility of this potential transposon, (ii) the role of IS*Ecp1B* in mobilizing the adjacent β-lactamase gene, and (iii) the role of IS*903D*. Different recombinant plasmids were constructed by inserting PCR products that encompassed either one or both ISs that bracketed the $bla_{CTX-M-19}$ gene in plasmid pILT-3 into the EcoRV site of plasmid pACYC184 (Table 2) and then transformed into *Escherichia coli* reference strain DH10B. Recombinant plasmids were constructed with the pACYC184 backbone to prevent overexpression of the IS*Ecp1B* transposase. Plasmid pC-1 contained the sequences of the potential transposon identified in plasmid pILT-3, whereas the following constructs lacked the right part of it (Fig. 1). Plasmid pC-2 possessed the IS*Ecp1B* sequence but lacked part of IS*903D*, and plasmid pC-3 possessed the entire IS*903D* sequence and a truncated IS*Ecp1B* transposase gene (Fig. 1; Table 2). *E. coli* RR1023 (pOX38) was transformed by electroporation with these recombinant plasmids (Table 2) (9). Plasmid pOX38 is a self-conjugative and IS-free plasmid. Transposition events were searched between inserts of several recombinant plasmids and recipient plasmid pOX38 after 24 h of growth in Trypticase soy broth. Transfer of the recombinant plasmids with the pOX38 backbone into streptomycin-resistant *E. coli* reference strain DH10B (Table 2) was then performed by conjugation as described previously (20), and transconjugants were selected on agar plates containing 100 μg of amoxicillin per ml (plasmid resistance marker) and 20 μg of streptomycin per ml (chromosomal marker). Several amoxicillin-resistant transconjugants were extracted and sequenced in part.

Susceptibility to chloramphenicol was always observed in transconjugant strains, thus ruling out full integration (chloramphenicol resistance) of the recombinant plasmids derived from pACYC184 into plasmid pOX38. Detection of IS*Ecp1B* and IS*903D* was performed using two sets of primers (primer ISEcp1A [Table 2] in combination with primer ISEcp1B [5′-TTTCCGCAGCACCGTTTGC-3′] and primer IS903A [5′-CA

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TABLE 1. Sequences identified as IRR boundaries for IS*Ecp1B*-mediated transposition

Description of sequence	Nucleotide sequence (5′→3′)[a]	No. of base pairs identical to perfect IRR[b]	Size of transposed fragment (bp)
IRL of IS*Ecp1B*	GATTCTACGTCAGT		
Deduced perfect IRR of IS*Ecp1B*	ACGTAGAATCTAGG		
IRR of IS*Ecp1B*	ACGTGGAATTTAGG	12	
IRR1 at the potential transposon end of pILT-3	GCGTTGATTCCTGG	9	4,798
IRR2 in plasmid pACYC184	GCATGGATTGTAGG	9	4,726
IRR3 in plasmid pBK-CMV	CCACAGAATCAGGG	9	4,541
IRR4 in the *tnpA* gene of IS*903D*	GATGAACTGCGGCG	3	3,273
IRR5 in the *iron* gene of pILT-3	GATGTGGAACCTCG	4	3,817
IRR6 in the *iron* gene of pILT-3	GCACCGGGATGGCG	3	4,167
IRR7 in the *iron* gene of pILT-3	GCGCCGCCGCGGGG	6	4,273
IRR8 in the *iron* gene of pILT-3	GGGGTCGAACTGGG	6	4,283

[a] Underlined nucleotides are nucleotides identical to those identified at the same positions in the deduced IRR of IS*Ecp1B*.
[b] A perfect IRR contains 14 bp.

CATGAAATCATCTGCGC-3′] in combination with primer IS903B [Table 2], respectively).

Amoxicillin-resistant transconjugants were not obtained when *E. coli* RR1023 (pC-3) lacking IS*Ecp1B* (Fig. 1) was used as a donor, suggesting that the IS*903D* element located downstream of the β-lactamase gene did not mobilize this latter gene. On the other hand, amoxicillin-resistant transconjugants were obtained when *E. coli* RR1023 (pC-1) and *E. coli* RR1023 (pC-2) were used as donors with a transposition efficiency of ca. 10^{-7} per donor. These results indicated that IS*Ecp1B* was essential for movement of the $bla_{\text{CTX-M-19}}$ gene.

Using *E. coli* RR1023 (pC-1) as a donor, all amoxicillin-resistant transconjugants except one contained IS*Ecp1B* and IS*903D* elements, with the $bla_{\text{CTX-M-19}}$ gene and DNA sequences being located on the right part of IS*903D* in plasmid pILT-3 (Fig. 1). Surprisingly, none of the 11 transconjugants that were sequenced had the entire potential transposon identified in natural plasmid pILT-3. Nevertheless, the transposition mechanism mediated by IS*Ecp1B* was evident, since 5-bp duplications were observed at the insertion sites in plasmid pOX38 (see below). These data suggested that the IRR sequences recognized by IS*Ecp1B* were located inside the potential transposon. Analysis of the boundaries of the transposed fragments identified sequences, similar to that of the IRR of IS*Ecp1B* and defined as IRR-like sequences, that had been recognized as such by the transposase of IS*Ecp1B* during a mobilization process.

Five different IRRs (IRR4 to IRR8) (Table 1) were identified in five amoxicillin-resistant transconjugants that were analyzed, four of them being located in the gene encoding the iron transport protein and one of them being located in the *tnpA* gene of IS*903D* (Table 2; Fig. 1). The number of identical base pairs among the sequence defined as IRR varied from three to six, corresponding to less than 50%.

Analysis of *E. coli* RR1023 (pOX38) transconjugants resistant to amoxicillin, obtained with plasmid pC-2 as a donor, and thus lacking the sequence used as IRR in pILT-3, also showed that transposition had occurred using nearby sequences of pACYC184. The IRR-like sequences bracketing the transposed fragment in these transconjugants remained unchanged.

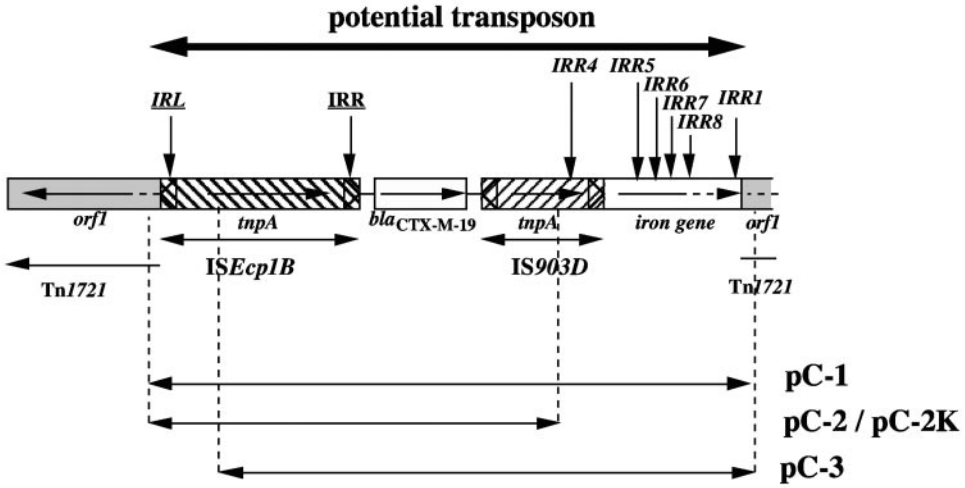

FIG. 1. Schematic map of the potential transposon structure in natural plasmid pILT-3 of *K. pneumoniae* ILT-3 (13). Open reading frames and genes are shown as boxes with an arrow indicating the orientation of each coding sequence and the gene name shown under the corresponding box. IRL and IRR motifs are indicated by vertical arrows. The IRL and IRR extremities of the IS*Ecp1B*-mobilized fragments are in italics, whereas those of the IS*Ecp1B* element are underlined. The sequences cloned in recombinant plasmids are indicated by double-headed arrows with the corresponding recombinant plasmid names.

TABLE 2. Strains and plasmids used in this study

Strain or plasmid	Relevant genotype or phenotype	Source or reference
Strains		
E. coli RR1023	pOX38 *recA56*; susceptible strain	9
E. coli DH10B	*araD139* Δ(*ara leu*)*7697 deoR endA1 galK1 galU nupG recA1 rpsL* F′ *mrcA* Δ(*mrr-hsdRMS-mrcBC*)ϕ80d*lacZ*ΔM15 Δ*lacX74;* streptomycin resistant	Stratagene
K. pneumoniae ILT-3	Extended-spectrum cephalosporin-resistant isolate	18
Plasmids		
pOX38	55 kb, F derivative, conjugative	9
pACYC184	Chloramphenicol resistant	New England Biolabs
pILT-3	Natural ca. 50-kb plasmid of *K. pneumoniae* ILT-3 producing β-lactamase CTX-M-19	18
pC-1	Recombinant plasmid pACYC184 with a 4,877-bp PCR fragment (primers PreA [5′-GCAGGTCTTTTTCTGCTCC-3′] and PreB [5′-TTTCCGCAGCACCGTTTGC-3′]) encompassing the potential transposon identified in pILT-3	This study
pC-2	Recombinant plasmid pACYC184 with a 3,635-bp PCR fragment (primers PreA and IS903B [5′-CCGTAGCGGGTTGTGTTTTC-3′]) containing IS*Ecp1B,* $bla_{CTX\text{-}M\text{-}19}$ gene, and truncated IS*903D*	This study
pC-2K	Recombinant plasmid pBK-CMV with the same 3,635-bp PCR fragment inserted into pC-2	This study
pC-3	Recombinant plasmid pACYC184 with a 4,606-bp PCR fragment (primers ISEcp1A [5′-GCAGGTCTTTTTCTGCTCC-3′] and PreB) containing $bla_{CTX\text{-}M\text{-}19}$ and IS*903D* but lacking part of IS*Ecp1B*	This study

They shared 9 of 14 bp of the perfect IRR sequence (Table 1) and corresponded to a segment belonging to the cloning vector. In order to rule out that the specific sequence of pACYC184 may play a role in this transposition process, the same inserted PCR product of pC-2 was cloned into the SmaI site of pBK-CMV, giving rise to pC-2K (Table 2; Fig. 1). Analysis of several amoxicillin-resistant transconjugants obtained with plasmid pC-2K as a donor also showed that transposition had occurred by involving sequences of pBK-CMV. The IRR-like sequences bracketing the transposed fragment in these transconjugants were always the same and shared 9 of the 14 bp of a perfect IRR (Table 1). These results indicated that sequences of pACYC184 (IRR2) or of pBK-CMV (IRR3) might be used as IRRs by IS*Ecp1B*, but further repeated experiments are needed to assess this hypothesis. The reason why the IRR1 of the potential transposon of pILT-3 was not the boundary of the transposed fragments in these in vitro experiments remains intriguing. One explanation could be that IRR2 and IRR3 share greater nucleotide identity in their 3′ extremities (7 and 8, respectively, of last 10 bp) to a perfect IRR than IRR1 (only 6 of last 10 bp).

No consensus sequence could be determined by comparing these 14-bp-long IRR sequences of recombinant plasmids and the IRR sequences of IS*Ecp1B* in pILT-3 (Table 1). Nevertheless, a guanosine residue located at the 3′ end of these IRRs was always found, likely indicating that this nucleotide was necessary in the transposition process. In addition, the last two nucleotides of these IRR sequences were either GG or CG. These data indicated that IS*Ecp1B* was involved in a transposition process with weakly related IRRs.

Analysis of the insertion sites of IS*Ecp1B*-mediated transposition revealed variable sequences in recombinant plasmids (AAGAA, TATAA, TATTA, GCTGA, ATATC, TAATA, TTTAC, AATGA, TTATA, ATAAG, and TGATT). No consensus sequence was identified among the 5-bp duplicated sites, whereas an A+T-rich content that may target IS*Ecp1*-mediated transposition as observed for IS*1* was noticed (16, 27). In silico analysis of six IS*Ecp1*-like sequences associated with $bla_{CTX\text{-}M}$-like or bla_{CMY}-like β-lactamase genes available in the GenBank databases did not identify target site duplications just bracketing the insertion sites of IS*Ecp1*-like sequences. This result likely indicates that after insertion, IS*Ecp1*-like elements may be responsible for mobilization of the adjacent sequences. In addition, this in silico analysis confirmed that the 5-bp sequences adjacent to the IRLs and IRRs of IS*Ecp1* elements were always A+T rich.

IS*Ecp1B* may use a wide range of DNA sequences as IRRs during a mobilization process that involves its adjacent sequences, therefore explaining its ability to mobilize structurally unrelated β-lactamase genes. Similar observations have been made for IS*1247* of *Xanthobacter autotrophicus*, which also belongs to the IS*1380* family (25). A single copy of IS*1247* may mobilize sequences located at its right-end extremity in what is considered a one-ended transposition process since only a single IS is involved.

These results may also be compared to those obtained with other IS elements, such as IS*91* and IS*911*, that are responsible for mobilization of adjacent sequences by a one-ended transposition mechanism (4, 22). In addition, transposition of other elements belonging to the Tn*3* family, such as Tn*21*, generates variable endpoints as a consequence of one-ended transposition (1, 15). This peculiar process is the consequence of recognition of several different IR sequences by the Tn*21* transposase, generating novel joint molecules (14). However, in our case, some homology between the different IRRs used by IS*Ecp1B* is noticed, whereas one-ended transposition (for example, with Tn*21*) requires one fixed end and another random one.

In the present study, we have demonstrated the ability for IS*Ecp1B* to mobilize the $bla_{CTX\text{-}M}$ β-lactamase gene. In addition, the IS*903D* element present in the potential transposon in pILT-3 had no role in the mobilization process.

In conclusion, it remains to be determined why the emerging expanded-spectrum β-lactamases of the CTX-M type that are

structurally related to several chromosomally located β-lactamase genes of enterobacterial species are associated specifically with IS*Ecp1*-like sequences.

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